STRAIN SPEC SHEET AMB-1

Magnetospirillum Magneticum

STRAIN OVERVIEW

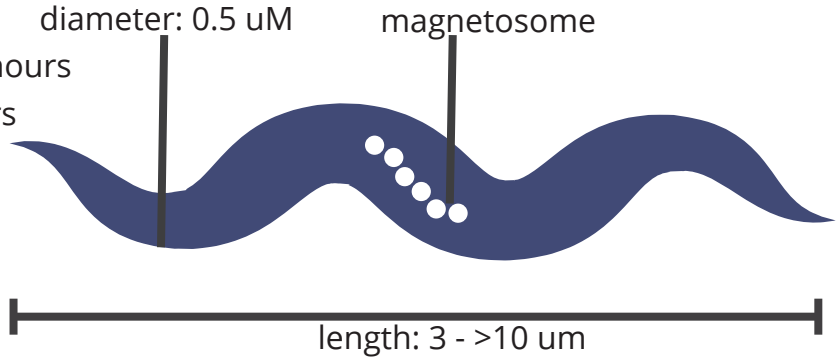
Magnetospirillum magneticum AMB-1 naturally takes in iron to form magnetosomes, a membranous organelle that allows the organism to align with a magnetic field. This organelle helps the strain navigate towards optimal, microaerophillic environmental conditions. By varying culturing conditions, researchers are able to control magnetosome formation. Researchers have used magnetic bacteria to determine magnetic poles in meteorites and rocks, to synthesize gold nanoparticles, and to serve as nanorobot. Potential applications include removal of heavy metal and radionuclides, enzyme immobilization, and oxygen depletion measurement.[1]

ATCC 700264

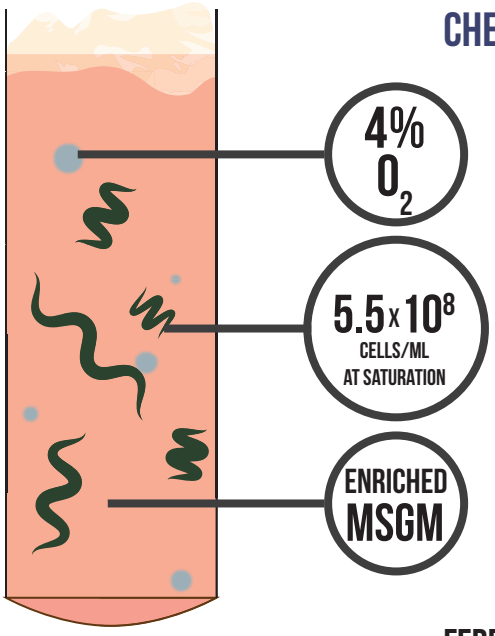
PUBMED 2250

1 BIOSAFETY LEVEL

MORPHOLOGY: spiral
DOUBLING TIME ON PLATES: 8 hours
COLONY FORMATION: 48 hours



CULTURING



30°C
OPTIMAL TEMPERATURE

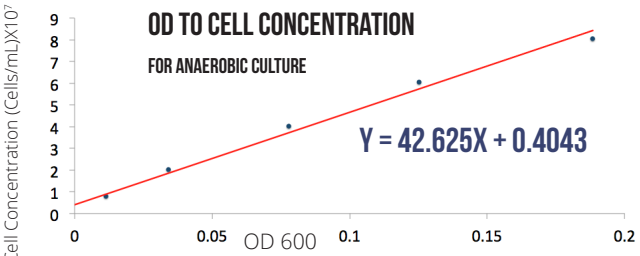
CHEMICALS FOR ENRICHED MAGNETIC SPIRILLUM GROWTH MEDIA (E-MSGM)

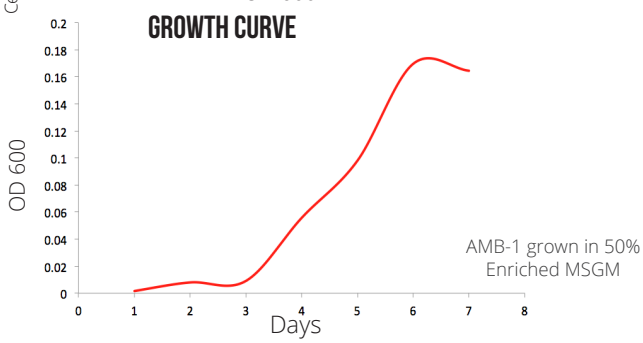
- 90% total volume milliQ water
- L-ascorbic acid 0.035 g/L
- Sodium acetate 0.07 g/L
- Sodium thiosulfate 0.1 g/L
- Sodium nitrate 0.12 g/L
- Succinic acid 0.37 g/L
- L-tartaric acid 0.37 g/L
- Potassiu phosphate monobasic 0.68 g/L
- Wolfe's Mineral Solution 5 mL/L [ATCC #MD-TMS]
- Yeast extract 0.1 g/L
- Polypeptone 0.2 g/L
- L-cysteine 0.05 g/L

pH adjusted to 6.9 with 10M NaOH

FERRIC MALEATE SUPPLEMENT NEEDED FOR MAGNETOSOME FORMATION.
Iron (III) chloride 0.486 g/L + DL-Malic acid 1.207 g/L
Prepare with milliQ water to make 100X stock solution.

QUANTIFYING CELLS





PLATING TECHNIQUES

For agar plates, add 10.0 g of agar per liter of E-MSGM. Add 1% of total volume E-MSGM in Wolfe's Vitamin Solution [ATCC #MD-VS] and 1% of total volume E-MSGM in iron malleate solution.

METHOD 1

Bacteria can be plated on 1% agar directly, as done for E. coli. Plates must be wrapped using an excess of Parafilm M® to reduce oxygen content in environment. Colony formation in 20-24 hours.

METHOD 2

Cells can be plated on 1% agar idish and grown in an anaerobic chamber. Colony formation in 10-12 hours.

TRANSFORMATION

NOTE: AMB-1 MUST BE GROWN AEROBICALLY PRIOR TO TRANSFORMATION TO PREVENT MAGNETOSOME FORMATION. MAGNETOSOMES WILL CAUSE CELLS TO DIE DURING ELECTROPORATION. SIMPLY INNOCULATE 500 UL CELLS IN 5 ML OF E-MSGM SUPPLEMENTED WITH 50 UL OF IRON MALLEATE AND 50 UL OF VITAMIN SOLUTION IN CULTURE TUBE ON SHAKER AT 30°C. IN 48 HOURS, CELLS WILL BE CONCENTRATED ENOUGH FOR TRANSFORMATION.

PARTS FOR VECTORS

ORI + REP GENE

Size of essential region: 2,973 bp
Extracted from pMGT.[2]
Available through Penn iGEM.

PMSP3

Size: 475 bp
Promoter available as BBa_K624016.

PMSP3 RBS

Size: 31 bp
Available as BBa_K624012.

Promoters of different strengths also available through BioBrick Registry.

PREPARING CELLS

Combine 10 tubes (3.5 mL each) of aerobically grown bacteria into a 50 mL falcon tube. Count cells and determine concentration. Must be at magnitude of at least 10^7 cells/mL to proceed. Centrifuge at 3700 RPM for 15 minutes. Pour off supernatant. If supernatant is murky, spin for longer. Resuspend pellet with 25 mL of 10 mM TES buffer containing 272 mM sucrose (pH 7.5). Centrifuge at 3700 RPM for 10 minutes. Resuspend pellet in total volume of TES+sucrose buffer needed to concentrate cells to 10^9 cells/mL. Split into 50 µL aliquots. Store at -80°C.[2,3]

ELECTROPORATION

Place 0.1-cm electroporation cuvette on ice. Add 1.5 µL of DNA to 50 µL aliquot of competent cells. Stir gently with pipet. Transfer solution to electroporation cuvette. Add 500 µL TES + sucrose buffer to an Eppendorf tube as recovery media. Electroporate with a Gene Pulser® (Bio-Rad Laboratories, Richmond, Calif.) at following settings: Capacitance 25 µF // resistance 200 Ω // 1mm cuvette // 10 kV/cm (1000V). Transfer 55 µL of electroporated solution into Eppendorf tube with recovery media. Recover at 30°C in a shaker at 100 RPM for 16 hours before plating.[2,3]

SAVING CELLS

Inoculate 500 µL cells into a sterile 50 mL falcon tube (1:100 dilution) with E-MSGM supplemented with 1% Wolfe's Vitamin Solution and 1% iron malleate. Grow 50 mL culture for 48 hours at 30°C (oxygen depletion in full falcon tube is sufficient). Centrifuge at 3500 RPM for 10 minutes. Make glycerol stock with final glycerol concentration of 30%.

[1] Yan, Lei, Shuang Zhang, Peng Chen, Hetao Liu, Huanhuan Yin, and Hongyu Li. "Magnetotactic Bacteria, Magnetosomes and Their Application." Microbiological Research 167.9 (2012): 507-19.
[2] Okamura, Y., H. Takeyama, T. Sekine, T. Sakaguchi, A. T. Wahyudi, R. Sato, S. Kamiya, and T. Matsunaga. "Design and Application of a New Cryptic-Plasmid-Based Shuttle Vector for Magnetospirillum Magneticum." Applied and Environmental Microbiology 69.7 (2003): 4274-277. Web.
[3] Schultheiss, D., Schüler, D. "Development of a genetic system for Magnetospirillum gryphiswaldense." Archives of Microbiology 179.2 (2003): 89-94. Web. doi: 10.1007/s00203-002-0498-z

SPECIAL FEATURES